Exhibit 5

1: Peptides 1997;18(4):551-7

Several receptors mediate the antisecretory effect of peptide YY, neuropeptide Y, and pancreatic polypeptide on VIP-induced fluid secretion in the rat jejunum in vivo.

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Several Y receptor subtypes have been cloned and/or pharmacologically characterized that mediate the effects of the regulatory peptides peptide YY (PYY), neuropeptide Y (NPY), and pancreatic polypeptide (PP). These peptides possess antisecretory properties on the intestine. This effect can be blocked in vivo by neural antagonists, suggesting the intervention of neural receptors, although epithelial PYY-preferring receptors have been evidenced on jejunal crypt cells. The purpose of the present experiments was to compare the antisecretory properties in vivo of a series of PYY and NPY derivatives with various affinities for different Y receptor subtypes, in order to determine which subtypes were involved. A model of VIP-stimulated secretion by rat jejunal loops was used. The results were compared with the binding affinities for PYY-preferring receptors determined on rat jejunal crypt cell membranes. Full-length PYY(1-36) was about three times more potent than NPY(1-36), and 10 times more potent than PP in the low dose range. PP, however, had a low efficacy limited to about 50% inhibition of VIP effect. Both Y1 agonists ([Leu31, Pro34]PYY and [Leu31,Pro34]NPY), and Y2 agonists [C-terminal fragments ranging from PYY (3-36) and NPY(3-36) to PYY(22-36) to NPY (22-36)] displayed potent antisecretory properties. PYY derivatives and fragments were always more potent than their respective NPY counterparts. In contrast, Y1 derivatives and PP had very low affinity for the epithelial PYY receptor as measured in vitro by radioreceptor assay. These data suggest that the antisecretory effect of PYY/NPY/PP peptides in vivo involves the effects of several receptors: a Y2-like, PYY-preferring receptor identical to the epithelial receptor, a Y1-like receptor, and a third receptor with high affinity for PP.

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1: J Med Chem 2000 Sep 7;43(18):3420-7

Structure-activity studies including a Psi(CH(2)-NH) scan of peptide YY (PYY) active site, PYY(22-36), for interaction with rat intestinal PYY receptors: development of analogues with potent in vivo activity in the intestine.

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Peptide YY (PYY) is a gut hormone that inhibits secretion and promotes absorption and growth in the intestinal epithelium. We have performed structure-activity studies with the active site, N-alpha-Ac-PYY(22-36)-NH(2), for interaction with intestinal PYY receptors. Investigation of aromatic substitutions at position 27 resulted in analogues that exhibited potent in vitro antisecretory potencies with N-alpha-Ac-[Trp(27)]PYY(22-36)-NH(2) exhibiting even greater potency than intact PYY. In vivo studies in dogs revealed that this analogue also promoted intestinal absorption of water and electrolytes during continuous intravenous and intraluminal infusion. Investigations carried out to identify features that would enhance stability revealed that incorporation of Trp(30) increased affinity for PYY receptors. A "CH(2)-NH" scan revealed that incorporation of reduced bonds at position 28-29 or 35-36 imparted greater receptor affinity. In general, disubstituted analogues designed based on the results of single substitutions exhibited good receptor affinity with N-alpha-Ac-[Trp(27),CH(2)-NH(35-36)]PYY(22-36)-NH(2) having the greatest affinity (IC(50) = 0.28 nM). Conservative multiple substitutions with Nle-->Leu and Nva-->Val also imparted good affinity. An analogue designed to encompass most of the favored substitutions, N-alpha-Ac-[Nle(24,28),Trp(30),Nva(31), CH(2)-NH(35-36)]PYY(22-36)-NH(2), exhibited a proabsorptive effect in dogs comparable to, but longer lasting than, that of intact hormone. Selected analogues also exhibited good antisecretory potencies in rats with N-alpha-Ac-[Trp(30)]PYY(22-36)-NH(2) being even more potent than PYY. However, the potencies did not correlate well with the

PYY receptor affinity or the proabsorptive potencies in dogs. These differences could be due to species effects and/or the involvement of multiple receptors and neuronal elements in controlling the in vivo activity of PYY compounds. PYY(22-36) analogues exhibited good affinity for neuronal Y2 receptors but poor affinity for Y1 receptors. Also, crucial analogues in this series hardly bound to Y4 and Y5 receptors. In summary, we have developed PYY(22-36) analogues which, via interacting with intestinal PYY receptors, promoted potent and long-lasting proabsorptive and antisecretory effects in in vivo models. These compounds or analogues based on them may have useful clinical application in treating malabsorptive disorders observed under a variety of conditions.

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1: J Surg Res 1999 Apr;82(2):151-5

Peptide YY inhibits growth of human breast cancer in vitro and in vivo.

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BACKGROUND. Hormonal manipulation is important in the treatment of breast cancer. Gastrointestinal hormones may have antiproliferative effects on malignancies arising outside the gastrointestinal tract. Peptide YY (PYY) suppresses growth of, and levels of, intracellular cyclic adenosine monophosphate (cAMP) in pancreatic adenocarcinoma. We hypothesized that PYY would inhibit growth of breast cancer. MATERIALS AND METHODS. MCF-7 human breast infiltrative ductal carcinoma cells in 96-well plates were treated with PYY at 1.25 pmol/ml. Control wells received an equal volume of bovine serum albumin to mimic experimental conditions. In vitro survival was determined by MTT assays, which reflect cell viability by measuring mitochondrial NADH-dependent dehydrogenase activity. MCF-7 cells in six-well plates were treated with PYY or albumin as described above. Intracellular cAMP levels in cell lysates were determined with a tritiated cAMP assay. One million MCF-7 cells were injected into mammary fat pads of 20 female athymic nude mice. Pellets releasing PYY at 400 pmol/kg/h were placed subcutaneously in 10 mice 24 h prior to cell inoculation. Tumors were harvested after 21 days, weighed, and measured with vernier calipers. RESULTS. PYY reduced in vitro growth by 40% ($P < 0.001$). Intracellular cAMP levels in PYY-treated cells were 62.4% less than those of controls ($P < 0.001$). Tumors from control mice weighed twice as much as those from PYY-treated mice ($P < 0.006$); volume of PYY-exposed tumors was one-third that of controls ($P < 0.005$). CONCLUSIONS. PYY inhibits growth of breast cancer in vitro and in vivo and may be of benefit in the treatment of this malignancy. The reduction in intracellular cAMP levels may contribute to the observed suppression of cell proliferation. Copyright 1999 Academic Press.

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1: Pancreas 1992;7(5):595-600

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Evidence for a direct inhibitory effect of PYY on insulin secretion in rats.

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Peptide YY (PYY) has been shown to inhibit stimulated insulin secretion under in vivo conditions in the mouse, the rat, and the dog. In the present study, we investigated the effects of PYY on insulin secretion from the isolated perfused rat pancreas and isolated rat islets. In isolated pancreas perfused in presence of 8.3 mM glucose, PYY at 10(-10) and 10(-9) M, but not at 10(-8) M, inhibited insulin secretion. In the presence of 5.5 mM glucose, PYY (10(-9) M) did not modify basal insulin release but reduced the biphasic insulin response to arginine (10 mM). PYY also markedly reduced the pancreatic vascular flow rate; this effect was observed at all three concentrations tested in a dose-dependent manner. In isolated islets, glucose (15 mM)-stimulated insulin secretion was inhibited by PYY at 10(-7) M. We conclude that in the perfused rat pancreas, PYY inhibits insulin secretion and induces vasoconstriction without a causal relationship. In addition, our results on isolated islets suggest that the inhibitory action of PYY on insulin secretion is exerted through a direct islet action.

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